PATENT
Case 51882AUSM1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:
Richard HORUK	:
Serial No. 09/915,411	: **Group Art Unit 1653**
Filed July 25, 2001	: **Examiner Anand U. Desai**

Title: NON-PEPTIDE CCR1 RECEPTOR ANTAGONISTS IN COMBINATION WITH CYCLOSPORIN A FOR THE TREATMENT OF HEART TRANSPLANT REJECTION

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 CFR § 1.131

Sir:

I, the undersigned, being duly warned, declare the following:

1. The following experiment, which was duly recorded in the attached final report and in the accompanying data spreadsheet (Exhibit A), was conducted upon my request under the supervision of Dr. Carol Clayberger at the Stanford University School of Medicine. The experiment recorded in Exhibit A was performed prior to January 2000.

2. The experiment recorded in Exhibit A was conducted to determine whether a therapeutically effective amount of a non-peptide CCR1 receptor antagonist and a sub-nephrotoxic amount of the immunosuppressive drug cyclosporin A may be beneficial in the treatment of heart transplant rejection in mammals.

3. The details of the experiment recorded in Exhibit A are as described in Example 4 of the instant application. Briefly, hearts from donor rats were transplanted into recipient rats, which were then treated with vehicle, non-peptide CCR1 receptor antagonist, cyclosporin A, or a combination of non-peptide CCR1 receptor antagonist and cyclosporin A. The amount of non-peptide CCR1 receptor antagonist administered was a therapeutically effective dose, and the amount of cyclosporin A administered was either a sub-nephrotoxic dose or a therapeutically effective dose. Heart allografts were palpated daily to assess graft function.

Vascularized hearts from donor ACI rats were heterotopically transplanted into the abdomen of recipient Lewis rats. End-to-side anastomoses were made from the ascending aorta of the donor heart to the abdominal aorta of the receipient and from the donor pulmonary artery to the recipient inferior vena cava, after ligation of the donor heart vena cava and pulmonary veins. Recipient rats were given either: 40% cyclodextrin s.c. three times per day ("*Con*"); 50 mg/kg of a non-peptide CCR1 receptor antagonist in 40% cyclodextrin s.c. three times per day ("*BX*"); cyclosporin A in olive oil by gavage 10 mg/kg once per day for four days ("*CsA10*"); cyclosporin A in olive oil by gavage 2.5 mg/kg once per day for the duration of the study ("*CsA2.5*"); cyclosporin A in olive oil by gavage 10 mg/kg once per day for four days plus 50 mg/kg non-peptide CCR1 receptor antagonist in 40% cyclodextrin s.c. three times per day ("*CsA10-BX*"); or cyclosporin A in olive oil by gavage 2.5 mg/kg once per day for the duration of the study plus 50 mg/kg non-peptide CCR1 receptor antagonist in 40% cyclodextrin s.c. three times a day ("*CsA2.5-BX*"). The transplanted hearts were evaluated daily by palpation. Rejection was deemed complete when palpable ventricular contractions ceased.

4. The results obtained are as follows. The mean allograft survival time of recipient rats given only the non-peptide CCR1 receptor antagonist was 8.8 ± 1.2 days compared to 6.8 ± 0.8 days for vehicle-treated rats (compare "*BX*" and "*Con*" in the table in Exhibit A, page 1). The mean allograft survival time of recipient rats given the non-peptide CCR1 receptor antagonist was statistically significant ($p = 0.0048$, by Breslow-Gehan-Wilcoxon analysis). The mean allograft survival time of recipient rats given a sub-nephrotoxic dose of 2.5 mg/kg cyclosporin A was 7.3 ± 0.5 days compared to 17.5 ± 5.9 days for rats on the same protocol additionally treated with the non-peptide CCR1 receptor antagonist (compare "*CsA2.5*" and "*CsA2.5-BX*"). The mean allograft survival time of recipient rats given a therapeutic dose of 10 mg/kg cyclosporin A was 12.9 ± 0.7 days compared to 18.4 ± 5.4 days for rats on the same protocol additionally treated with the non-peptide CCR1 receptor antagonist (compare "*CsA10*" and "*CsA10-BX*"). The mean survival times of recipient rats treated with either 2.5 or 10 mg/kg cyclosporin A plus the non-peptide CCR1 receptor antagonist were statistically significant from the mean survival times of rats treated with either 2.5 mg/kg or 10 mg/kg cyclosporin A alone ($p = 0.0009$ and $p = 0.0148$, respectively). The raw data, including the number of days of graft survival, the mean number of days of graft survival per group, the standard deviation, and the number of rats per group, is tabulated in Exhibit A, page 2. The cumulative survival plot is depicted in the figure in Exhibit A, page 1 (and in Figure 4 of the instant application.

n-0519

5. The results shown above demonstrate that the invention – significant prolongation of heart transplant survival time in mammals by combined treatment with a therapeutically effective dose of a non-peptide CCR1 receptor antagonist and a sub-nephrotoxic dose of cyclosporin A – was reduced to practice prior to January 2000.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issuing thereon.

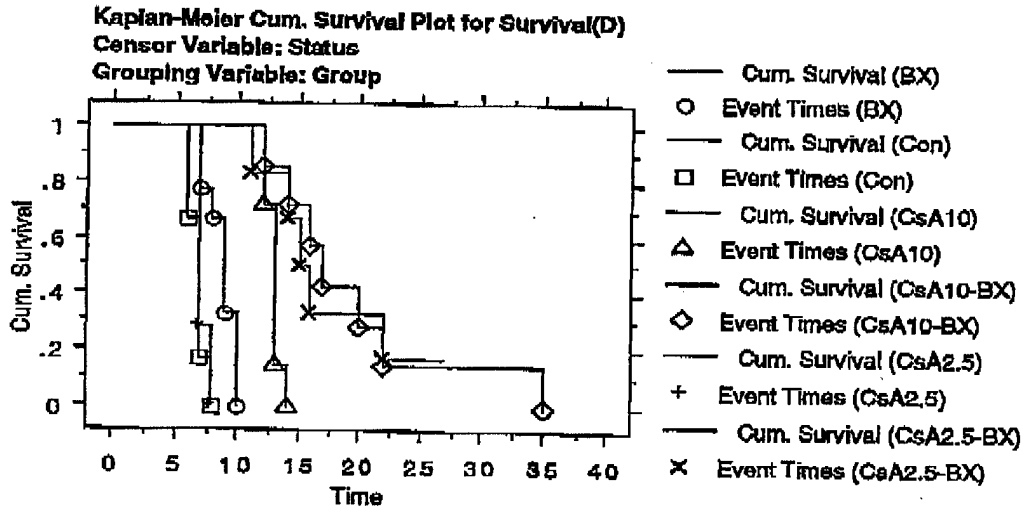
12/11/03
Date

Richard Horuk

n-0519

Exp-82 Final Report

ACI to LEW BX in Cyclodextrin 50mg/Kg Tid SC (Ref: bi02233-85A, & bi02233-96A)
CsA2.5: CsA .25mg/Kg PO QD For forever
CsA10: CsA 10mg/Kg PO QD X 5 (POD 0 to 4)
CsA10-BX: CsA 10 QD X 5 + BX SC Tid X forever
CsA2.5-BX: CsA 2.5 QD + BX SC Tid X forever
Con: coming from Exp-76 (Cyclodextrin 2ml/Kg, SC, Tid)
BX: coming from Exp-76 [BX (Ref: Bi02233-45A) 50mg/Kg, SC, Tid]



		Con	BX	CsA 2.5	CsA 10	CsA 2.5-BX	CsA10-BX
	Mean(D)	6.8	8.8	7.3	12.9	17.5	19.4
	SD (D)	0.8	1.2	0.5	0.7	5.9	7.4
	N	6	9	7	7	6	7
	Con	N/A	N/A	N/A	N/A	N/A	N/A
	BX	0.0048	N/A	N/A	N/A	N/A	N/A
P:	CsA 2.5	0.2116	0.0125	N/A	N/A	N/A	N/A
	CsA 10	0.0006	0.0004	0.0004	N/A	N/A	N/A
	CsA 2.5-BX	0.0014	0.001	0.0009	0.0443	N/A	N/A
	CsA 10-BX	0.0006	0.0004	0.0004	0.0148	0.6683	N/A

P was counted by Breslow-Gehan-Wilcoxon.

Exp-82 data

Group	Survival(D)	Status	Mean(D)	SD(D)	N	Exp-82 ACI to LEW BX in Cyclodextrin 50mg/Kg Tid SC
CsA2.5	7		0			(Ref: bl02233-85A, & bl02233-96A)
CsA2.5	7		0			CsA2.5: CsA 25mg/Kg PO QD For whole Exp
CsA2.5	7		0			CsA10: CsA 10mg/Kg PO QD X 5 (POD 0 to 4)
CsA2.5	8		0			CsA10-BX: CsA 10 QD X 5 + BX SC Tid X forever
CsA2.5	7		0			CsA2.5-BX: CsA 2.5 QD + BX SC Tid X forever
CsA2.5	7		0			Con: coming from Exp-76 (Cyclodextrin 2ml/Kg, SC, Tid)
CsA2.5	5		0	7.3	0.5	7 BX: coming from Exp-76 [BX (Ref: bl02233-45A) 50mg/Kg, SC, Tid]
CsA10	13		0			
CsA10	13		0			
CsA10	12		0			
CsA10	14		0			
CsA10	13		0			
CsA10	13		0			
CsA10	12		0	12.9	0.7	7
CsA10-BX	12		0			
CsA10-BX	35		0			
CsA10-BX	22		0			
CsA10-BX	20		0			
CsA10-BX	16		0			
CsA10-BX	14		0			
CsA10-BX	17		0	13.4	7.7	7
CsA2.5-BX	14		0			
CsA2.5-BX	27		1			
CsA2.5-BX	22		0			
CsA2.5-BX	15		0			
CsA2.5-BX	16		0			
CsA2.5-BX	11		0	17.5	5.9	6
Con	6		0			
Con	7		0			
Con	7		0			
Con	7		0			
Con	8		0			
Con	8		0	6.8	0.8	6
BX	10		0			
BX	9		0			
BX	10		0			
BX	7		0			
BX	7		0			
BX	9		0			
BX	10		0			
BX	8		0			
BX	9		0	8.8	1.2	9